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SCHAFFER, S.

București, April 2, 1960

Dear Professor Lederberg,

I received your letter and thank you very much for the informations concerning the replica plating. I have sent you the paper Schäfler and Mintzer issued in Dokladî Akad. Nauk SSSR (Reports of Academy of Sciences URSS) 1959, 128, 830 which I hope you received meantime. I regret not to be able to send you our papers from the J. of Bacteriol., August, 1959 and February 1960 as I have not obtained reprints. As the article in Reports of Acad. of Sciences URSS appeared in Russian, I am sending you a short English summary.

It was found that there exists an optimum of lactose concentration for the mutation cellobiose$^+ \rightarrow$ lactose$^+$. For S. stanley, S. heidelberg, and S. glostrup the optimum is between 2,5 and 5%, and for S. minnesota 1,25%. It seems that the concentration influence is not based on a more rapid fermentation of lactose by the lact$^+$ variants but on their appearance in a greater proportion. Analogous results were obtained as well by the inoculation of the original strain S. glostrup in Koser's peptone medium with various concentrations of lactose.

Further investigations showed that in presence of great concentrations of lactose, the cellobiose positive variants possess a weak beta-galactosidasic activity which complicates the genetical interpretation of the results.

I should like to communicate to you some data concerning your strain E. coli K 12, substrains F$^-$ W677 and Hfr (H) which Prof. M. Ciucă received from Dr. Hayes (J. of gen. Microbiol., 1957, 16, 97).

The frequence and time of mutative fermentation of lactose by the W677 strain in fluid medium is influenced by the concentration of lactose and of the culture medium composition. In peptone water (Bakto Peptone Difco 1% + bromothymol blue 1/5000) the optimal concentration of lactose is 4-5%; the time of fermentation is reduced in average with 2-3 days in comparison with the 0,5% lactose concentration. In Koser's saline solution + 0,1% Yeast Extract Difco the optimal concentration of lactose is of 1,5 - 2%. In presence of optimal concentration of lactose in peptone water there are marked differences in the fermentation of the different tubes (4-11 days), whilst in Koser's

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medium + 0,1% yeast extract 90-95% of the tubes ferment between the 7th and 8th day. The observed phenomene may be partially explained by a) the enhancing of the fermentation of lactose by the Lac$^+$ mutants in presence of increased concentrations of lactose, b) the appearance of a great number of Lac$^+$ mutants which are slowly fermenting lactose (in which the fermentation is partially inhibited by aminoacids and peptones), and a smaller number of mutants which ferment lactose more actively (like the Hfr strain). Fermentation in Koser's medium + 0,1% yeast extract is mainly due to the first type of mutants and the fermentation in peptone water to the second type. The simultaneous appearance of both types makes this interpretation not to be absolute.

The mutative fermentation of salicin, arbutin, manitol and d-arabinose is accelerated in peptone water in comparison with Koser's medium + 0,1% yeast extract.

A pronounced influence of the culture medium was also observed in the appearance of the Lac$^+$ papillae. In Koser's solution + 2% agar, 1% lactose and 0,2% yeast extract the Lac$^+$ papillae appear after 5-6 days, in presence of 0,5% yeast extract after 3-4 days, with 1% yeast extract in 2-3 days (the culture is completely covered by papillae, of which the majority are slow fermenters); in presence of 2% yeast extract the number of papillae decreases and appears after 4-5 days, the active fermenting mutants are in greater proportion. In 5% yeast extract the appearance of papillae is practically inhibited as in nutritive agar + lactose. If the yeast extract is substituted with Bacto Peptone Difco the number of papillae is very reduced in presence of 0,2 and 0,5%, and a bigger number was obtained with 2% (4-5 days). Although the population in the Bacto Peptone 2% is of 55-60% of that of the yeast extract 1% the number of papillae is only of 5% compared to that of the yeast extract. As the concentration of 5% peptone, the complete inhibition of papillae is observed. With Bacto Casitone Difco we obtained similar results. High concentrations of Proteose Peptone Difco (5-7%) weakly inhibit the appearance of papillae. In EMB agar the papillae appears after 3-5 days, with a similar frequence as in 0,5% yeast extract. The addition of treonine, leucine and aneurine to Bacto Peptone Difco slightly activated the appearance of Lac$^+$ papillae.

In the mutative fermentation of salicin, arbutin, manitol and maltose the concentration of 2% yeast extract inhibits less the formation of papillae than in case of lactose; the latter can also appear in nutritive agar with the respective sugar but in a much lesser proportion. The concentration of 5% yeast extract completely inhibits the appearance of papillae. Similar observations were noticed on cellobiose fermentation by S.glostrup, where the unfavoureble influence of Bacto Peptone and nutritivr agar is less pronounced.

It seems to me that the direct relation between the number of mutants and the number of papillae is only valid in definite culture medium conditions, the appearance of papillae depending of the microsocological conditions of the respective medium. The fact is characteristic that in presence of 1% yeast extract a lesser number of Lac$^+$ papillae is obtained on isolated colonies than on the corresponding surface and population of the continous growth culture (condition in which the mentionned experiments were carried out).

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Contrary to certain other E.coli strains, the mutative fermentation of salicin and arbutin seem to be identical. The F^- strain shows a greater mutation rate of arbutin and salicin than the Hfr strain. In presence of 0,2-1% yeast extract the F^- strain yields many small papillae, whilst the Hfr strain yields few big papillae. In the F^- strain seems to exist a very weak primer fermentation, difficult to establish on account of the frequent apparition of the mutants. This primer fermentation is accentuated in some recombinations with the Hfr strain. The data obtained up to now, concerning the recombination between the Hfr and F^- strains, show that the recombinants Lac^+ presented different intermediary forms of the mutation arbutin $^+$ and salicin$^+$, whilst the Xyl^+ recombinants resemble in the majority of cases to the Hfr strain.

We have obtained in Koser's medium + 2% agar, 0,2 - 0,8% yeast extract and 1% maltose papillae Mal^+ which yield secondary mucoid giant colonies with a diameter up to 10 mm and 3-4 mm height. The appearance of these giant colonies is rather irregular, appearing on 2-3 plates out of 10 after incubation of 5 days at 37° and for 4 to 12 days at room temperature (15-19°). The phenotypical formation of mucus around all the Mal^+ papillae appears by incubation only at room temperature on 0,5% and especially 0,2% yeast extract. The frequency of secondary giant mucoid colonies seems to depend of the yeast extract sample. If the number of plates was sufficiently great, we obtained in all cases giant mucoid colonies. We have not obtained such colonies on EMB agar and Koser medium with 1 and 2% Bacto Peptone or Proteose Peptone.

Besides maltose fermentation the mucoid forms keep the characteristics of the strain W677 ($T, L^-, B_1^-, Lac^-, Xyl^-$, Manitol$^-$) The mutation rate versus salicin and arbutin resembles that of the Hfr (H) strain. The mucoid form is F^- and lesser mobile in fluid medium as the original strain. The mucoid mutants do not yield mucus in fluid medium as on yeast extract in absence of sugars, but forms mucus on nutritive agar. The mucus production is partially inhibited by glucose and is very active in presence of l-arabinose and trehalose. The mucus is more abundant at room temperature but appears also at 37°. These characters differenciate these mutants from those obtained by WOUST (J.Bacteriol., 1959, 77, 452) under the influence of the anti-serum. Through passages on nutritive agar and Dorset medium, the mucoid character is stable. The mutants can easily return to normal form in presence of salicin or arbutin, 40-60% of papillae salicin$^+$ and arbutin$^+$ being devoid of mucus.

By recombination of the mucoid mutant with the Hfr strain on minimal medium with maltose appear 5-10% mucoid colonies as much in the Lac^+ recombinants as in the Xyl^+ and Manitol$^+$ recombinants. In the majority of recombinants the mucoid character is more labile. According to me, the formation of mucus seems to be determined by a citoplasmatic factor related to the mutation Mal^+, but this hypothesis still demands an experimental verification.

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As I know many of your works concerning the K 12 strain (Genetics, J. of Bacteriol., 1957, Reports of the National Academy of Science, etc.) only from reviews, I would be highly interested in what measure some of the phenomena observed by us, correspond with your observations.

On account of some other preocupations, our investigations concerning the K 12 strain can be interrupted, nevertheless, in case of some supplementary data would interest you, I stand always at your disposal.

With my best regards,

yours sincerely

S. Schäfler